DOES BILE INHIBIT OR STIMULATE GROWTH OF THE COLON GROUP?[1]

By Max Levine[2]

When in 1906 Jackson suggested the use of lactose bile for isolation of the colon group, it was enthusiastically received by water analysts, for its superiority to glucose broth as a presumptive test was distinctly manifest, and in 1912 bile was adopted by the Committee on Standard Methods of Water Analysis.

The medium recommended consisted of undiluted ox bile (or 10 per cent dried bile) to which was added 1 per cent peptone and 1 per cent lactose. That this medium inhibited the growth of some strains of the colon group was clearly recognized by the Committee who believed, however, that only the attenuated or weakened individuals were affected and that consequently this selective antiseptic action was an added advantage. Thus they state, "Attenuated B. coli does not represent recent contamination and all B. coli not attenuated grow readily in lactose bile." . . . . "In the interpretation of the sanitary quality of a water it is best to discount the presence of attenuated B. coli and to be sure to obtain all vigorous types. The lactose bile medium accomplishes both these objects."

In 1913, Jordan, after a careful investigation, concluded that there is no relation between attenuation and the antiseptic action of the bile. He found that freshly isolated strains were inhibited to as great or ever a greater degree than old cultures. Cumming, in a comparison of lactose bile and lactose broth, records that with sewage preliminary enrichment in the former yielded only 25 per cent as many colon forms as were obtained with lactose broth, and that a similar study, with river water, gave only 50 to 70 per cent as many colon organisms as was obtained with the broth. From his study on the Potomac River, Cumming concludes that about

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one-half of the colon bacilli were lost when the bile medium was employed for preliminary enrichment. Obst obtained similar results.

In consequence of these reports the 1917 Standard Methods recommend lactose broth for preliminary enrichment and the presumptive test.

Opposed to the views expressed above is that of Hale who maintains that the bile medium is far superior, that formation of gas was more rapid, produced in larger amounts and that the anaerobic spore-formers (Cl. welchii) were less frequent. In a discussion of a paper by Winslow 1916, Hale states as follows:

> Since the Committee on the Revision of Standard Methods has advocated the use of lactose broth we have again made at Mount Prospect Laboratory a series of comparisons with the lactose bile and lactose broth, confirming by litmus lactose agar. The broth was made as recommended by the committee; the bile was 5 per cent as recently recommended from this laboratory. The results were all in favor of bile, quicker gas formation, gas in larger amounts, and less B. welchii forms. In one day the results with bile were practically equal to those obtained in two days with the broth.

Thus the question has been raised as to whether bile inhibits or stimulates growth of the colon group. The author felt that this query might be adequately answered and the supposedly conflicting views of Jordan and Hale reconciled by a study of the effect of various concentrations of bile. In this connection it should be pointed out that whereas Jordan, Obst and Cumming used the original whole (or 10 per cent dried) bile medium, Hale has decreased the concentration to 5 per cent dried bile.

The following report deals with the effect of various quantities of sodium taurocholate (Merck) and evaporated bile (Difco) on the rate of multiplication of Bact. coli and Bact. aerogenes.

The method of study was to inoculate $\frac{1}{100,000}$ cc. of a twenty-four-hour peptone culture into 10 cc. of 0.5 per cent peptone water containing definite concentrations of dried bile or sodium taurocholate, and after seven hours' incubation in a water bath at 37°C. to determine the number of viable cells by planting on agar (37°C.—forty-eight hours). The results are indicated in the following tables.

It is apparent that for both Bact. coli and Bact. aerogenes there exists an optimum concentration of bile constituents. Sodium taurocholate up to 1.5 per cent accelerated growth of Bact. aerogenes; the maximum count being obtained with a concentration of 0.75 per

TABLE 1

Effect of concentration of sodium taurocholate (Merck) on the growth of Bact. aerogenes in 0.5 per cent peptone (Difco)

TIME	PER CENT OF SODIUM TAUROCHOLATE	AVERAGE NUMBER BACTERIA PER CUBIC CENTIMETER	GENERATION TIME
Series no. 1			
minutes			*minutes*
0		206	
420	0 (Control)	605,000	36.4
420	0.25	2,120,000	31.5
420	0.5	3,165,000	30.2
420	0.75	4,300,000	29.1
420	1.0	4,150,000	29.3
420	1.5	3,100,000	30.2
Series no. 2			
0		223	33.0
420	0 (Control)	1,490,000	33.1
420	0.25	1,425,000	33.2
420	0.5	1,540,000	32.9
420	0.75	2,830,000	30.8
420	1.0	2,450,000	31.3
420	1.5	2,260,000	31.5

TABLE 2

Effect of concentration of sodium taurocholate (Merck) on the growth of Bact. coli in 0.5 per cent peptone (Difco)

TIME	PER CENT OF SODIUM TAUROCHOLATE	AVERAGE NUMBER BACTERIA PER CUBIC CENTIMETER	GENERATION TIME
Series no. 1			
minutes			*minutes*
0		255	
420	0 (Control)	1,240,000	34.2
420	0.25	980,000	35.2
420	0.5	1,780,000	32.9
420	0.75	1,582,000	33.3
420	1.0	1,390,000	33.8
420	1.5	220,000	43.1
Series no. 2			
0		235	
420	0 (Control)	460,000	38.4
420	0.25	4,150,000	29.7
420	0.5	4,020.000	29.8
420	0.75	4,635,000	29.4
420	1.0	4,330,000	29.6
420	1.5	3,940,000	29.9

TABLE 3

Effect of concentration of evaporated bile (Difco) on the growth of Bact. aerogenes in 0.5 per cent peptone

TIME	PER CENT BILE	AVERAGE NUMBER BACTERIA PER CUBIC CENTIMETER	GENERATION TIME
	Series no. 1		
minutes			*minutes*
0		112	
420	0 (Control)	1,300,000	31.1
420	0.5	2,145,000	29.5
420	1.0	2,450,000	29.1
420	2.0	820,000	32.7
420	5.0	230,000	38.1
420	10.0	9,000	66.3
	Series no. 2		
0		136	
420	0 (Control)	642,500	34.4
420	0.5	715,000	33.9
420	1.0	930,000	32.9
420	2.0	595,000	34.7
420	5.0	307,000	37.6
420	10.0	1,300	128.9

TABLE 4

Effect of concentration of evaporated bile (Difco) on the growth of Bact. coli in 0.5 per cent peptone

TIME	PER CENT BILE	AVERAGE NUMBER BACTERIA PER CUBIC CENTIMETER	GENERATION TIME
	Series no. 1		
minutes			*minutes*
0		222	
420	0 (Control)	3,240,000	41.7
420	0.5	6,675,000	38.7
420	1.0	8,350,000	37.8
420	2.0	8,050,000	27.7
420	5.0	1,000,000	32.2
420	10.0	280,000	41.8
	Series no. 2		
0		252	
420	0 (Control)	3,200,000	30.8
420	0.5	6,070,000	28.8
420	1.0	8,450,000	27.9
420	2.0	8,950,000	27.7
420	5.0	1,900,000	32.6
420	10.0	280,000	41.2

cent of the salt. Similar (though more irregular) results were obtained with Bact. coli.

The effect of Difco evaporated bile was particularly marked and distinct. The maximum count of Bact. aerogenes was obtained with a concentration of 1 per cent dried bile. Higher concentrations were distinctly inhibitory. Thus in the presence of 10 per cent dried bile, which was the medium originally recommended, the count was only a fraction of a per cent of that obtained with peptone water alone.

Bact. coli grew best in the presence of 2 per cent evaporated bile and the concentration of even 5 per cent was not detrimental but 10 per cent was markedly inhibitory.

In conclusion, it may be said that the value of bile media in routine water analyses is still an open question and needs further careful study. From the results here presented it seems that the inclusion of a small quantity of dried bile (1 to 2 per cent) for preliminary enrichment and presumptive test media for the colon group would be highly desirable. For comparable results a standard evaporated bile or pure bile salts are of course essential. The advantages of such a medium are (1) growth of Bact. coli and Bact. aerogenes is accelerated, (2) many of the anaerobic spore-forming lactose-fermenters are inhibited and (3) the sporing lactose fermenters, capable of growing aerobically, do not grow in peptone lactose bile.

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